SCIENTIFIC STUDY ON BIODIVERSITY AND PHYSICO-CHEMICAL ANANLYSIS OF TOP SOIL OF COAL MINING AREA IN JHARKHAND.

A PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

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CERTIFICATE

This is to certify that the research project report entitled **"Scientific study on Biodiversity and physico-chemical analysis of top soil of coal mining area in Jharkhand."** submitted by **Miss Payal Naresh Sakhare** in partial fulfillment of the requirements for the award of the degree of Master of Technology in Biotechnology and Medical engineering with specialization in Biotechnology at the National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in the report has not been submitted to any other University/Institute for the award of any Degree or Diploma.

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DECLARATION

The present study entitled **"Scientific study on Biodiversity and physico-chemical analysis of top soil of coal mining area in jharkhand."** is based on my original research work and no part of the thesis has so far been submitted for the award of degree in Master of Technology in Biotechnology or any other degree or diploma to the **NIT Rourkela,** Orissa, India or elsewhere.

Place: Rourkela, Odisha
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ABSTRACT

Soil is a system, in which continuous interface between minerals and microorganisms control the physico-chemical and biological properties of ecosystem. Anthropogenic actions such as mining activities have resulted in radical alternations in their geochemical cycles and often lead to land degradation. For this purpose the present study was conducted on physico-chemical analysis and microbial diversity of top soil and water collected from the coal mining area. Physico-chemical analysis of soil indicates that the soil is slightly basic in nature. The bulk density and specific gravity of the soil samples were found to be very low, indicating that the soil is rich in organic matter which is essential for the growth of the plants. The chloride content of soil is low in range between 0.006 to 0.021 mg/g, whereas the phosphorus content is in the range of 0.025 to 0.005 mg/g which is found to be low from the normal range. The sulphur content ranges from 0.067 to 0.01 mg/g. Five bacterial isolates (Aeromonas spp., Corynebacterium spp., Neisseria spp., Staphylococcus spp., Lactobacillus spp.) and one fungal species (Aspergillus spp.) were identified from the top soil and water samples of the study area. Biochemical tests were performed and from the obtained results, presence of diverse group of microorganisms was confirmed in soil samples that also suggest presence of essential macro and micro nutrients for the growth of plants as well microorganisms in soil. Along with microbial diversity floral diversity of mining area was also studied and finally mitigation measures has been suggested for the preservation of floral diversity, the loss of which was assessed for mining activity to be carried out.

Keywords: *Mining, biodiversity, flora, Bacteria, Morphology, Biochemical tests.*

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Abbreviations

Cm	-	Centimeter
^{o}C	-	Degree centigrade
et al.	-	And others
G	-	Gram
ha.	-	Hectare
hrs.	-	Hours
HCl	-	Hydrochloric acid
H_2O_2	-	Hydrogen peroxide
M	-	Meter
Mg	-	Microgram
Ml	-	Microlitre
Mg	-	Milligram
Ml	-	Millilitre
Mm	-	Millimeter
mM	-	Millimolar
M	-	Molar
NA	-	Nutrient agar
N	-	Normal
P_2O_5	-	Phosphorous pentoxide
K_2O	-	Potassium dioxide
NaCl	-	Sodium chloride
Km^2	-	Square kilometer
H_2SO_4	-	Sulphuric acid
K_2HPO_4	-	Dipotassium phosphate
KH_2PO_4	-	Monopotassium phosphate
KNO_3	-	Potassium nitrate
UV	-	Ultra Violet
Wt.	-	Weight
w/v	-	Weight by volume

CHAPTER I

INTRODUCTION

Biodiversity is molded by natural activities and progressively, by the impact of people. It structures the web of life of which we are an integral part and where we so completely depend upon biodiversity. The importance of present need has been shifted towards biodiversity consequences for environment capacities (BDEF), where variation amongst the communities is a driving behind diversity in ecosystems [1]. The world now recognizes that the loss of biodiversity contribute to worldwide climatic changes. Woodlands are the principle mechanism for the change of carbon dioxide into carbon and oxygen. The loss of woodland spread, coupled with the increase of carbon dioxide and different gasses through industrialization contribute to the 'green house effect. Global warming is the dissolving ice tops, bringing about an ascent in the ocean level which will submerge the low lying ranges on the planet. It is bringing on significant air progressions, prompting expanded temperatures, serious droughts in a few zones and sudden floods in different regions [2]. Biological diversity is additionally vital for saving biological methods, for example, altering and reusing of supplements, soil formation, dissemination and purging of air and water, worldwide life help (plants retain CO_2, give out O_2), keeping up the water adjust inside environments, watershed insurance, keeping up stream and waterway streams all around the year. The conservation of "biodiversity" is along these lines necessary to any method that points at enhancing the nature of human life [3].

Biodiversity is combatively examined as one biological system property deciding stability and may subsequently a key for human prosperity. Microorganisms represent the essential backbone of any biological system, and it is fundamental to understand their reaction under changing abiotic and biotic conditions. Consequently, diversity–stability connections in microbial groups require closer consideration. Here we address this issue by considering different stability measures of microbial benefit as a capacity of genotypic wealth and practical

differences, two of the most unmistakable indices of biodiversity [4]. Soil microorganisms assume key parts in biological communities and impact an expansive number of vital environment courses of action, including supplement securing, nitrogen cycling, carbon cycling and soil establishment. In addition, soil organisms represent the unseen share in soil and embody a large part of the genetic diversity on Earth. Free-living microorganisms likewise help the maintenance of plant diversity through their impact on the accessibility of distinctive structures, both organic and inorganic, in soil. Microbial differences can additionally push plant diversity and profit when microorganisms associated with diverse plant species or when distinctive organisms give distinctive assets [5]. This differing quality relies upon the wide variety of plants, creatures and microorganisms. Microorganisms in soil assume significant parts in different biogeochemical cycles (BGC) and also responsible for the cycling of organic compounds. These microorganisms impact over the ground biological communities by helping plant nutrition, plant wellbeing, and soil structure and soil ripeness. All living beings in the biosphere rely upon microbial diversity in that bacteria are crucial for the keeping supplements and for replenishing nutrients over the ground biological communities [6]. Biological community groups are controlled by abiotic and biotic requirements on species concurrence and predominance, where the principle logical target is to understand the regulation and support of diversity. The great interest on microbial biodiversity is to understand microorganisms, for that we have to know what is there and what we can utilize. Investigations of related living beings may yield potential items. Bacterial and fungal metabolites may be source of new compound and pharmaceutical items. Different pharmaceuticals such as mevinolin, which lessens cholesterol in people, have been found through microbial screening. Fungi are a possibly rich resource of useful medicinal compounds. Microorganisms, particularly chemolithotrophic microorganisms e.g. Thiobacillus ferroxidans,

and T.thiooxidans, are progressively utilized within mining for controlled step bioleaching of metals [7].

Objectives of the present study

The present study was aimed with the following objectives:

1. Scientific study on the physical and chemical characteristics of the top soil and water collected from coal mining area.
2. Study of the microbial diversity in the top soil and water of the coal mining area and identification of the micro flora by various biochemical tests.
3. Study of the floral species in the mining site.
4. To suggest mitigation measures for floral diversity in the coal mining area.

Chapter 2

REVIEW OF LITERATURE

Biological diversity deals with the nature's variety in the biosphere. This variety observed at three extents: (a) The variety of species within community, (b) the genetic variability within a species and (c) the organization of species in an area into different plants and animals community which represents ecosystem diversity. The diversity of life of all three organizational level genetics, species and ecosystem is being disturbed by anthropogenic activities [8].

2.1 Significance of Biodiversity

In spite of its thin layer, the structure of atmosphere, sediments and soils substantially are affected by the biosphere. Human welfare directly hinge on or are influenced by other organisms for nutrition, health (favorable pathogens, symbionts, organisms providing medicinal substances and diseases living inside the human body or on the human body) and habitat (like building materials and clothing). This mutuality between the specific elements of environmental systems and the interactions between various types of complexes, called "biocomplexity", takes into record that the individual parts of environmental complexes provide very less data about the behavior of the systems. Modifying pathways of energy and material flow (C-, N-cycles) may influence ecosystem variables directly by functional groups of organisms and even single species, or regulation of these flows altered indirectly by a biotic condition. Furthermore, the "information flow" of biodiversity is driven by some ecosystem variables itself, e.g. extent of pollination, pests and diseases (e.g. pest control by antagonistic interactions and host species dilution, invasion control by niche occupation) [9].

2.2 Threats to biodiversity

The rate of loss of biodiversity and species extinction increased rapidly due to human

actions. Human activities including introduction of exotic species, habitat loss, biogeochemical cycles, changes in climate and anthropogenic actions like mining, pollution and over harvesting threaten biodiversity in many ways [10]. The reason behind biodiversity loss is the natural habitat conversion to other land uses. Deforestation has resulted in guaranteed impacts on its prosperous and distinctive biodiversity. Depletion of nutrients of soil and erosion are due to conversion of land to agricultural use which has more fatal impacts. With increase in disturbance of forest through logging processes, urbanization or agriculture including birds, mammals, butterflies, ants, bees, moths there is decrease in species richness and population density [11].

2.3 Mining

Mining is the wrenching out or excavation of precious minerals from the earth which forms the mineralized package of economic interest to the miner. It is the activity which is related with the extraction of minerals [12]. Mining process requires the removal of soil during the extraction and transport of minerals. Waste removal and their characterization is the major portion in mining process. The process of mining produces significant influence on the environment during mining and years later. Thus due to this there should be mitigation measures adopted.

2.3.1 Coal mining

The motive of coal mining is to acquire coal from the ground. Electricity generated by Coal is worth for its energy and it is extensively used. Coal is used as fuel by steel and cement industries. Different types of coal have been used like steam coal mostly used in power generation, metallurgical coal used in steel production. Other important uses of coal are in chemical and pharmaceutical industries, gasoline solvents, wood preservatives, copper, iron and

lead smelting. Activated carbon used in filtration of water, carbon fibers used in construction and tennis rackets [13]. Coal mining has progressed due to digging, tunneling, and manually extraction of coal on carts to large open cuts and long wall mines over recent years from early days. Coal has great importance over worldwide

It is estimated that there are over 984 billion tons of coal reserves in world. Availability of Coal reserves are in almost every country worldwide, with retrievable reserves in around 70 countries. The biggest reservoirs are in the USA, Russia, China and India. The size, location, and properties of most coal reserves of countries are seen after centuries of mineral exploration thus, quite well known. Asia is the huge market for coal, which is currently holds for 54% of the global consumption of coal , although China is responsible for most prominent portion of this. Most of the countries do not have adequate energy needs are fulfilled by natural energy resources, and therefore they need to give energy to assist meet their requirements [14].

Coal reservoirs bring to light through exploration actions. The production of geological map of the area is the activity usually involves in the coal mining, then bringing out geochemical and geophysical surveys, followed by investigation drilling. This allows toconstruct prominent picture of the area.

Table 1. Top coal producers worldwide

Top ten hard coal producers			
China	3471 Mt	Russia	334Mt
USA	1004 Mt	South Africa	253 Mt
India	585 Mt	Germany	189 Mt
Australia	414 Mt	Poland	139 Mt
Indonesia	376 Mt	Kazakhstan	117 Mt

2.3.2 Types of coal resources

The classification of coal is usually based upon their gradational properties. Gross calorific value and fixed carbon are calculated and classified according to the mineral matter free basis of coal. According to fixed carbon on the dry basis, the higher coal ranks are classified and the gross calorific value on the basis of moisture, the lower rank coals are classified accordingly. The ranking criteria are based on the properties of the maceral (carbonaceous) material used, and the effects of variable mineral matter contents are eliminated, which are unrelated to rank. Peat is considered as progenitor of coal, lignite or brown coal is the lowest rank coal, sub-bituminous coal ranges between those of lignite and of bituminous coal, bituminous coal is a heavy sedimentary rock, usually black but sometimes dark brown in color with bands of very bright and dull material, Anthracite is a highest rank of coal which is harder, it is black glossy coal, graphite is strictly the highest rank coal and is difficult to ignite so cannot be used as fuel [15].

Table 2: Classification of coals by rank, ASTM system

Class	Fixed carbon		Volatile		Matter energy
	Dry (%)	Moist(%)	Dry(%)	Moist(%)	Moist (mj/kg)
1. Anthracite	> 98–86	> 92–81	< 2–14	< 2–15	35.5–31.4
2. Bituminous	86–54	81–45	14–57	13–40	35.8–24.4
3. Sub-Bituminous	55–53	45-37	53–55	36–38	26.7–19.3
4. Lignite (brown coal)	52	32–26	32–35	38–50	< 19.3

2.3.3 Coal mining methods

Coal mining is largely divided into two methods, one is opencast mining or surface

mining and other is deep mining or underground mining. Mining methods depends on the geology of the coal deposits which determines the choice. The mainly effective method is underground mining which holds about 60% of coal production in the world. Several countries are pursuing surface mining process which is contemplate to be main mining process [16].

2.3.3.1 Surface mining

When coal appears almost near to the surface, it can be economical to withdraw the coal by open cut mining methods. strip mining is typically used which reveal the coal by the development of a moving strip or open pit. The earth on the coal is recognized as overburden. A strip of overburden subsequently to the formerly mined strip is then drilled. The drill holes are packed with explosives and are blasted. The overburden is then dismissed using large earthmoving instruments such as shovels, draglines, and excavator or bucket wheels, trucks and conveyors. This overburden is kept into the formerly mined strip. When all of the overburden is eliminated, the underlying coal will be exhibited as a strip which is known as a block. Coal in the blocks are instructed and blasted or ripped and stuffed onto trucks and conveyors for shipping to a washing plant or crushing. A new strip is generated next to it once all coal is departed [17]. Open cast coal mining retrieves a greater amount of the coal reserve than underground method. Globally, around 40 percent of the coal is manufactured by surface mining. Surface mining holds for almost 80 percent of Australia's manufacture and two-thirds of United States production. many square kilometers are covered by Opencast coal mines. Scraping off the topmost portion of the mountain that involves the exposure of the underlying coal is a type of surface mining. It is highly disputable because of the extreme changes in the covering of streams, topography, the creation of hollow fills, and the disturbance of ecosystem. Terrace mining is used where the

overburden is too thick to allow waste dumping directly over the pit, it is necessary to use intermediate cyclic or continuous shipping (e.g. conveyors or trucks) to relocate the overburden to where it can be ripped back into the formerly mined void. The whole mine make move over the ore reserve from one end to the other because it is the multi-benched sideways-moving method, but not necessarily in a single bench. The purpose of the excavation depth and type of machinery used are the number of benches usually used [18].

2.3.3.2 Underground mining

The two fundamental methods of mining coal underground are long wall mining and room & pillar mining. The proportionately flat coal beds typically of the United States, these two methods are well suited for extraction. Principally, this long wall mining is utmost simple. By excavating passageways a coal bed is packed out into a panel surrounding its perimeter. More than 1 million tons of coal is present in a panel , up to 80% of which to be retrieved.

The use of a sophisticated coal-shearing machine, self-advancing hydraulic roof supports, and an armored conveyor corresponding the coal face by long wall mining extraction is about a constant operation. The shearing machine drives on the conveyor which slits and spilled the coal onto the conveyor which works under the movable roof supports for shipping mine out of it. shear reverses the direction when it travel across the coal face of the full length, and travels back reverse parallel to the face, taking another cut. The support has been moved closer to the fresh cut face as the shear passes across each of the roof support. The workers and equipment has been located parallel to the face which are protected by the roof supporting steel canopies, while following the supports as they are advanced the roof is permitted to collapse. Extraction sustained in this way until the whole panel of the coal is separated [19].

2.3.4 Coal mining in India

The quick industrialization of the country has been subscribed significantly by coal which is a pre-eminent source of energy in India. The availability of relatively large quantity of coal reserves and inadequacy of other energy sources leads to the significance of coal in the energy basket of India. Coal presently accounts for 55% of India's complete energy utilization. India is presently the third largest producer of coal and contributes about 8% of the total coal production in the world (IBM 2012). Coal mining in India comprises a share of 80% in the whole mining process, with the rest 20% disseminate among different raw materials such as iron, lead, gold, copper, bauxite, zinc, etc. Chikkatur et al (2009) have approximated the coal reservoirs i.e. the resources that can be financially mined given the present technology and costs at only 44 billion tones. As stated by them, these reservoirs will last up to coming 30 and 60 years, to be contingent on the amount of domestic coal production. These quantity put in uncertainty that the notion of large quantity of coal reserves and create perturb and in doubt with regard to acceptability of coal supplies essential to meet the growing energy urge of India [20]. The coal mining industry in India is comparatively in recent development as compared to the European countries. Many numbers of miners have been brought from England during the early phase of coal mining in India. The coal mining in India is started in the first century, But in the direction of the end of the 19^{th} century, the coal in the jharia field in Jharkhand state are the largest reserves of high quality became increasingly perceived. T.W.H Hughes of the geological survey of India has been topographically investigated the area of jharia in 1865; however mining advancement was not genuinely consumed for number of years. By 1890, the aggregate tonnage from the field has surpassed million imprint and throughout the early years of the present century some ¾ million tons of coal were prepared yearly. Different coalfields in Bihar and Bengal, which were utilized

throughout the latter half of the nineteenth century, incorporated a little yield totaling by most accounts 7200 tons from Bengal, between 1896 and 1900 in bigger scale in Bihar [21].

India is the third biggest maker of coal in the world. Coal In India is recognized as the black gold. India has 2, 93,497 million tones of land assets of coal estimation from entire nation. India has high amount of ash content in the coal. The normal content of the ash in Indian coal is 35-38% for while foreign made coal powder content is 10-15%. In this respect, washing will assist to decrease the ash content by 7-8 percent [22].

Likewise, about whether the calorific quality and the ash content remains substance of thermal coals in India have crumbled as the better quality coal reserves are exhausted and surface mining and mechanization extended. This postures huge difficulties.

Transporting a lot amount of ash-producing minerals squanders vitality and makes deficiencies of rail autos and port offices. A low-quality, high-ash coal makes issues for power stations, incorporating disintegration in parts and materials, trouble in pulverization, poor emissivity and fire temperature, low radioactive exchange, and excessive measures of fly powder holding a lot of unburned carbons [23]. Indian coal is described by the accompanying quality aspects like:

(i) Lower to mid-range grade coal

(ii) Ash amount is high

(iii) Moisture amount is low

(iv) Sulfur amount is low

A wide range of coal use, extending from power generation to steel generation to base and business utilization, the nature of coal might be enhanced by washing. Coal washing is, no doubt advertised in India as various studies have indicated that calorific quality of washed coal is higher than unwashed coal, deciphering into good power generation effectiveness. The low ash

amount of washed coal brings about easier outflows too. India will setup 20 new washeries with yearly limit of 111 Million Tones to ease better acknowledge of its produce [24].

2.3.5 Coal mining in Jharkhand state of India

Jharkhand was represents on 15th.november 2000 as the 28th State of the Republic of India. It imparts its limits to Bihar in the North, Orissa in the South, West Bengal in the East and Chhattisgarh and Utter Pradesh in the West. It remains between latitude $22^{o}00'$ and $24^{o}37'$ North and longitude $83^{o}15'$ and $87^{o}01'$ East. The geographic territory of the State is 79,714 sq.km, which is 2.4% of the nation's aggregate geographic zone. It incorporates the area which is overwhelmingly tribal crowded. It comprises principally of Chhotanagpur level, which is a piece of the Deccan Biographic area. The general geology of the State is undulated and brimming with hillocks and levels. The State of Jharkhand has an introduced power producing limit of 1390 MW as against the national limit of 1,05,000 MW. The thermal power producing limit in the State is 1260mw and is 24% of the national limit. Within a brief period of time, the Government of India may want to supplement thermal power production with hydel and atomic force. Coal mining likewise began 100 years back [25].

Jharkhand has about 40 % of the country's mineral assets, for example, coal, iron metal, copper, uranium, mica, bauxite, stone, limestone, silver, graphite, magnetite and dolomite. Woodlands and forests possess more than 29% of the state, making it one among the states with more stupendous woods spread. Jharkhand has around 40% of the country's mineral riches. The state is one of the biggest makers of coal, mica and copper in India. In view of its vast mineral holds, mining and mineral extraction is the real business in the state. Jharkhand's economy has developed at something like 9.3% between 1999-2000 and 2008-09. The state gives speculation

open doors in areas, for example, mining and metal, power, foundation, assembling and nourishment processing [26]. The State of Jharkhand has huge natural difficulties. Its mineral riches had been and are constantly discriminately uncovered. Coal mining and beneficiation is may be a standout amongst the most contaminating activities. 66% of coal of the nation dwells in this state. Coupled with this, there are various thermal power plants which produce fly ash as waste. Change of iron metal mixed with coal into sponge iron is one of the most dirtying assembling procedures. Jharkhand has 32% of iron metal store of the nation. Something like 20 assembling units of diverse limits are in operation in the state with an aggregate introduced limit of 3500t for every day and a lot of people more will mushroom in the late future. Amongst the different modern areas, the incorporated iron and steel plants help a significant heap of toxins to nature from their subunits, in particular, coke broiler, recalcitrant, sintering, steel softening and hostage power plants. Jharkhand represents 29% of mineral riches in the non-coal division [27]. Additionally coal and press mineral, a portion of the vital minerals show in the state are – bauxite, chromites, copper mineral, lime stone, dolomite, manganese metal, mica, quartz, silica sand, pyrite, feldspar and bentonite, separated from uranium and a lot of more minerals. Mineral based commercial enterprises are air contaminating in nature. However, water is needed in a mineral based industry, for cooling, extinguishing, transforming, evaporator, robust squanders transfer and so on, the gushing from a portion of the areas does not experience any noteworthy change in terms of the water quality and could be reused 100%, yet frequently, and they are squandered. Mineral wastes produce 30-50% solid wastes which are of concern [28].

2.3.6 Coal mining and its impact on environment

Our utilization of coal energy has drastic effect on nature. Minimizing the effect of human activities on the natural environment is the essential matter. However coal makes a critical

commitment to the social and money improvement around the world, its ecological effect has been a challenge.

Coal mining, especially surface mining includes temporary disintegration of land. This expands number of natural issues, including soil disintegration, dust commotion, water contamination and effects on nearby biodiversity. Steps are taken in present day mining procedures to minimize these effects. Mitigation measures minimizes the effect of mining on the natural environment and serves to ensure the biodiversity [29].

2.3.6.1 Land disturbance

Before coal mining activity various conditions and potential issues must be characterized to minimize the effect of mining on the ground and surface water, soils, nearby land use, local vegetation and wild life populations.

2.3.6.2 Mine subsidence

An issue related with the underground coal mining is collapsing, while the ground level brings down as an after effect of coal has being mined underneath. Thus an intensive investigation of subsidence examples permits impacts of underground mining on the surface has to be quantified. This verifies the protected and most extreme recuperation of a coal asset while offering security to the next area utilization [30].

2.3.6.3 Water pollution

Acid mine drainage (AMD) is a metal-rich water made from the compound reaction between water and rocks containing sulfur. The overflow shaped is normally acidic and as often

as possible hails from areas where coal mining exercises have display rocks holding pyrite, a sulfur-bearing mineral. This corrosive run-off release substantial heavy metals, for example, copper, mercury, lead into ground and surface water. There are mine administration systems that can lessen the complexity of Acid Mine Drainage, and powerful mine configuration can be keep water far from corrosive creating materials and help avoid Acid Mine Drainage [31]. Dynamic administration includes introducing a water medication plant, where the AMD is first mix with lime to kill the corrosive and afterward passes through settling tanks to uproot the silt and particulate metals to Passive treating has been focus to create a progressing in the direction of oneself framework which can treat the effluent without steady human intervention.

2.3.6.4 Dust and Noise pollution

Throughout mining process, the effect of air and noise contamination on the laborers and nearby localities might be decreased by modern mine procedures and specific equipment. Dust amount could be overseen by spreading water on roads, stockpiles and transports. Dust gathering frameworks and supplementary area encompassing the mine act as a buffering zone between mine and its neighbor. Trees planted in these buffering areas can additionally diminishes the visual effect of mining process on nearby groups [32]. Dust pollution can be guided via careful supply, protection and sound enclosure around hardware.

2.3.6.5 Rehabilitation

Coal mining is a transitory utilization of land, so it is important to restore the area once mining activities have been stopped. In practice a definite restoration or recovery plan should be maintained and approved for each coal mine, the period from the begin of methodology until the

mining has completed. Land recovery is a major part of current mining processes around the globe and restoring the area. [33]. Mine reclamation processes are embraced gradually with the casting and forming of ruin heaps, restoration of topsoil, seeding with grasses and plantation of trees occurring on the mined-out zones. Care should be taken to move streams, natural wildlife, and other significant assets. Recovered land can have numerous applications, including horticulture, forestry service, natural wildlife residence and recreation.

2.3.6.6 Soil erosion

Due to mining investigation there is extraordinary soil disintegration which leads to hindering to nature's domain. Numerous laborers working the area are oblivious of the natural effect that coal mining process and other mining processes has. They are not being aware of which strategies are best for nature's domain and can reduce soil disintegration. The principle sorts of soil disintegration in the mining zones, including exogenic methods, are water erosion and man-induced erosion, wind erosion. Water erosion happens in the rainy season which stretches out from June to September. Most soil loss in the regions is connected with water erosion, which incorporates sprinkle disintegration, surface disintegration and channel disintegration. Wind erosion, joined by dust storms, once in a while happens in the dry season that reaches out from January to April. Man-assisted disintegration is fundamentally co partnered with quickened disintegration from the diverse mine workings [34]. Geography and soil fertility has been changed or crushed because of burrowing of surface mines and dumping of overburden rock mass as large stacks. Because of mass deforestation in the mining territories soils have been exposed for further erosion. Indeed the soils which were prior evacuated for the mining and dumped somewhere else are exposed to the erosion and weathering.

2.3.6.7 Loss of biodiversity

Mining exercises has great influence on the biodiversity in the state, in the same way as soil fertility, animal creatures, birds, and plant species and so on. Unsustainable mining is the characteristic assets that have been a major element for devastation of biodiversity. Vegetation in the forest regions have been under steady danger as a result of the unsustainable misuse of the minerals. Open cast mining should have the most extreme effects on the ecology. In this framework, area is obliged for mining region as well as to dump the overburden rock mass.

The effects of mining on the nature are mentioned underneath.

- Removal of vegetation (flora) has made pressure on fauna to vacant the region needed for mining and different purposes.
- Dust in climate helped different activities which may hinder the growth of a portion of the plant species in encompassing regions.
- Noise and vibrations because of blasting, transportation and operation of the machines have headed out little creatures including wild animals and birds from adjacent forests.
- Due to the mining processes top soil has been harmed.
- Topography and situation has changed because of burrowing of open pits and dumping of the overburden weathered rock mass as extensive heaps [35].

Chapter 3

MATERIAL AND METHODS

The study was done on top soil and water collected from the mining area in jharkhand. Physico-chemical analysis of soil and water samples was carried out and their microbial diversity was also studied. Soil physical properties like surface, moisture content, particular gravity, bulk density were measured. Chemical properties of soil like alkalinity, pH, sulfur content, phosphorus content, chloride content, were likewise determined. Microbial diversity of the top soil and water was studied by different biochemical tests, for example, Gram's staining, MR-VP test, Citrate utilization test, Indole test, Catalase test, Nitrate test, Oxidase test, Urease test, Mannitol mortality test, Hydrogen sulfide gas generation test and Starch hydrolysis test.

3.1 Study Area

The present study was selected from coal mining areas in Jharkhand state, which must be having enriched carbon content in soil and water sources.

3.2 Sample collection

Field study was directed in the eight different geographical locations of coal mining area. Soil and water specimens were gathered from these areas in and around the proposed mining location by random sampling design method.

3.3 Analysis of soil samples

A soil auger was utilized to acquire samples with at least 0.5 kg of soil for every sampling zone. The main 0-10 cm of the soil samples was inspected. Specimens were obtained utilizing aseptic methods. Soil specimens were put in hard fixed plastic packs and kept at 4°C to keep them field moist and to save biotic properties. Soil dampness content was measured

gravimetrically in the after drying of the soil samples at 105°C. The physical and chemical investigation for the characterization of soil spread layers of the separate locality were carried out. The air dried topsoil samples were ground and pass through 2mm sieve. The collected topsoil samples in the sieving (2mm) were utilized for investigation of distinctive soil quality parameters. The accompanying strategies are briefly said underlined.

3.3.1 pH

The pH value which is a estimate of the hydrogen or hydroxyl particle action of the soil water framework shows whether the soil is acidic, neutral or alkaline in response. Crop development endures under low and in addition high pH. The instrument for pH estimation ordinarily utilized is an advanced pH meters have single cathode get together. The instrument being a potentiometer, the ph scale must be adjusted before utilization with buffer solutions of known pH values. 1 gm of soil is taken in a 50ml beaker and 10 ml of distilled water is mixed in it [36]. The suspension is mixed at general intervals for 30 min. and the pH is recorded. The suspension is blended well simply before the cathode are drenched and readings were taken.

3.3.2 Moisture content

The standard strategy for determining moisture content of soil is the oven-drying technique. This is the procedure prescribed for soil. Dampness content measured by gravimetric technique and communicated as percentage. Loss of weight of the specimens was calculated to focus the dampness content.

3.3.3 Bulk density

About 10 g of soil sample was dried in an oven at 105°C until a steady weight is accomplished. At that point somewhat dried soil was exchanged to a measuring chamber and the volume was recorded. At that point the weight of the volume was again measured utilizing a weighing balance [37].

Bulk density (g/cm3) = Weight of soil (g)/ Volume of soil (cm3)

Where 1 ml = 1 cm^3

3.3.4 Specific gravity

Something like 10 g of soil sample was dried in an oven at 105°C until a consistent weight is achieved. At that point a pre-weighed glass flask of known volume was loaded with the dried soil samples and its weight was recorded in a weighing balance machine. An alternate pre-weighed glass container of the same volume was loaded with distilled water and its weight was recorded [37].

Specific Gravity = (A2 – A1)/ (B2 – B1)

Where A2 is the weight of the flask with soil; A1- weight of vacant bottle utilized for soil; B2- weight of container with distilled water and B1- weight of empty bottle utilized for water.

3.3.5 Chloride content

About 10 gm of air-dry soil was taken and 100 ml of distilled water was added to make up a suspension of 1: 100 w/v. 10 ml of sample was taken in a flask and 5-6 drops of potassium chromate indicator was added to it. The shade of the sample got yellow. It was titrated against silver nitrate until a persistent brick red shade shows up at the end point (Jackson, 1958).

$$\textbf{Chloride (mg/l)} = \left(\frac{\mathbf{F}}{\mathbf{10}}\right)\left(\frac{\mathbf{V}}{\mathbf{W}}\right)\left(\frac{\mathbf{1}}{\mathbf{100-M}}\right)$$

Where, V= vol. of sample, W= weight of soil, M= moisture content of soil

3.3.6 Phosphorus content

In a 25 ml volumetric flask, 5 ml of the soil sample is added and including 5 ml of dickman and bray reagent. At that point neck of the volumetric is washed down and the substances are diluted to about 22 ml, then1 ml of dilute stannous chloride solution is included and volume is made up to the imprint. The intensity of the blue shade is measured (utilizing 660 nm) simply following 10 minutes and the concentrations of phosphorus are resolved from the standard curve (Jackson, 1958).

3.3.7 Sulphur content

Sulfate content of soil examples was measured by the method (Jackson, 1958). 10 g of air dried soil sample was included 100 ml of refined water to got 1:10 w/v suspension. This suspension was sifted through a filter paper (whatman paper No.44) and filtrate was acquired. 50 ml of this filtrate was taken in a conical flask and 10 ml of NaCl –HCl solution and 10 ml of glycerol- ethanol solution and 0.15g barium chloride was added. The last solution was mixed for one hour then absorbance was assumed on a spectrophotometer at 420nm against distilled water as blank, acquired absorbance qualities were contrasted and standard sulfate solution and sulfate content in samples were figured.

3.4 Microbial diversity

3.4.1 Microbial populations

Microbial diversity especially for bacteria and fungi were done for collected soil and water samples from coal mining area by following standard dilution plate procedure [38]. In this procedure, 1ml water sample or 1g of soil sample mixed in 10ml of sterile water from that 1 ml of mixed solution was taken and volume was made up to 100ml with sterile water which was further serially diluted to get 10^{-4} dilutions. From these diluted specimens, 1 ml water specimen was distributed over each of three replicates and after that media for development of diverse microorganisms were included and it is supplemented by agar utilized for isolation of microbes while potato dextrose agar and ammonium chloride-starch agar medium were utilized for fungi and actinomycetes individually, the petriplates were incubated at 35 °C for 48 h for bacterial growth and 25 °C for 72 h for fungi growth. The microbial population was enumerated as colony forming units (CFU) from a serial dilution of soil suspensions. The microbial colonies were measured in the three replica plates and the average values are then calculated. The population of microorganisms depends upon the dilution factor for each of the sample.

3.4.2 Isolation of Bacteria

The media utilized as a part of this examination was nutrient agar medium. 28g of nutrient agar powder was weighed and dissolved in the 1000 ml of distilled water. It was mixed vigorously and dissolved utilizing hot plate after which was sterilized in autoclave for 15 min at 121°C. It was then permitted to cool after which it was distributed in Petri dishes and permitted to solidify. Segments of the suspension were inoculated on the nutrient agar medium by streaking and were incubated at 37°C for 24h. After which colonies with clear zone of inhibition

were observed.

3.4.3 Gram's staining

Colonies which were develop on nutrient agar medium were gram stained as per standard gram staining methodology [39]. To study the Gram's staining i.e. Gram (+ve) or Gram (-ve) characters of the isolates, the cultures were taken and diluted suspensions of the microorganisms (8-12h old) were spread on the clean slides and air dried. The smear is then heat fixed by passing over a flame for 2-3 times. The slides were overflowed with cystal violet solution for 1 minute and then washed with water. The slide then flooded with Gram's Iodine for 1minute and then the slides were washed with water and decolorized it with 95% ethyl alcohol dropped from a dropper until no violet color was appeared on the slide. The slides were then washed with water and counter stained with safranin stain for something like 30 seconds and again washed with water. The slides were air dried and analyzed under a microscope utilizing 100x magnification utilizing daylight filter.

Composition of Gram's staining:

A. Crystal violet solution:

Solution I: Crystal violet (85%) dye 2 gm dissolved in 20ml of 95% ethyl alcohol.

Solution II: Ammonium oxalate monohydrate 0.2 gm dissolved in 20ml distilled water.

Equal parts of solution I with solution II were mixed and used for staining.

B. Gram's iodine:

1 gm iodine and KI 2 gm mixed in 300 ml of distilled water, stored in a brown bottle.

C. Safranin solution:

Safranin 2.5 gm in 100ml of 95% of ethyl alcohol. Add 10 ml of alcoholic solution with 100 ml water.

3.4.4 Sub Culturing

Bacterial isolates having demonstrated a cleared zone of inhibition on nutrient agar plates were sub cultured into nutrient agar slants for a brief time preservation and to purify the isolates. The microorganisms were inoculated in the nutrient agar slant utilizing a sterile wire loop and incubated at 37°C for 24hours. The slant test tubes containing the microorganisms were kept in refrigerator at 4°C for brief time storage before biochemical tests were ran on the isolates for identification.

3.5 Biochemical Tests for Bacterial Identification

Biochemical tests were accomplished according to standard procedure of Cappuccino [40].

3.5.1 Indole Test

This test is utilized to check capacity of the living microorganisms to produce indole from tryptophan or to locate the presence of enzyme tryptophanase which converts tryptophan to indole. One percent tryptophan stock in a test tube was inoculated with bacterial colonies. After incubating the test tubes at 37°c for 48hours, then one 1ml of chloroform was added to the broth. The test tube was shaken delicately, then Kovac's reagent was added within the broth and this was additionally shaken tenderly and permitted to remained for twenty 20 minutes. The formation of red coloration at the top layer showed positive and yellow coloration demonstrates negative.

Media Composition

Chemicals	Grams/litre
Peptone	20
NaCl	5
pH	7.4

3.5.2 Catalase Test

Presence of enzyme catalase which catalyzes breakdown of hydrogen peroxide into water and oxygen was examined over the slide overflowed with hydrogen peroxide solution. This was done by picking the bacterial colony on the slide and putting a drop of hydrogen peroxide on the slide with bacterial smear. Presence of bubbles shows positive response while absence of bubbles demonstrates negative response.

3.5.3 Citrate Utilization Test

Capability of the microscopic organisms to grow in a medium holding citrate as sole source of carbon and energy source is detected. Citrate usage is observed by appearance of growth and increment of pH from 6.8 which is shown by the change in color of bromothymol blue indicator of the medium. This was done by inoculating the test microorganism in test tube holding Simon's citrate medium and this was inoculated for 24 hours to 72 hours. The development of deep blue color after incubation shows a positive result. No growth and yellowish-green color of the slant showed negative result.

Media composition:

Chemicals	Grams/litre
$MgSO_4$	0.2
NaCl	0.5

$(NH_4)2HPO_4$	1
K2HPO4	1
Sodium citrate	2
Bromothymol blue	0.08
Agar	20

3.5.4 Methyl Red Test

The test is utilized to detect acid generation from glucose. generation of acid brings down the pH of the medium beneath 4.2 which is detected by the pH indicator which is methyl red solution. Microscopic organisms were inoculated into tubes holding methyl red (MR) stock and incubated at 30 ±0.1°C for 72 hours. Some little amount (2-3 drops) of methyl red solution was added in the test tubes. If red color developes after addition of methyl red implied a positive test while yellow shade meant a negative test.

Media composition:

Chemicals	**Grams/litre**
Peptone	7
Potassium phosphate (KPO_4)	5
Dextrose	5

3.5.5 Oxidase Test

To identify presence of the enzyme oxidase in the microorganisms was performed. It catalyzes transport of electrons between microorganisms and the redox dye which is methylene blue. Some drops of methylene blue were added to 72 hour culture in nutrient broth media. Positive response was demonstrated by change in colour of the stock to colourless in few seconds.

Reagent: 0.2% solution of methylene blue was mixed with distilled water.

3.5.6 Urease Test

In the presence of urease producing microorganisms urea splits into ammonia & CO_2 was detected. Urease broth was prepared and bacterial culture was inoculatedin the broth & incubated at 30°C for 72 hours. The test detects the presence of urease enzyme in the microorganisms.

The development of Purplish pink coloration of the medium indicated negative reaction.

Media composition

Chemicals	Grams/litre
Peptone	1
NaCl	5
K_2HPO_4	2
Glucose	1
Urea	20
pH	6.8
Phenol red	6

3.5.7 Voges-proskauer (acetoin production) test

Capacity of numerous microorganisms to ferment carbohydrates particularly glucose with production of acetyl methyl carbinol reduction product into neutral products and carbon dioxide rather than organic acid is evaluated. Microorganisms were inoculated into the test tubes holding VP stock and incubated at 30±0.1°C for 72 hours. After incubation a blended solution of α-napthol and potassium hydroxide were added to 2.5 to 5 ml of culture in the test tubes. presence of dark red color of the medium demonstrated positive result.

Reagent: 3ml of 5% α-napthol in absolute ethanol is mixed with 1ml of 40% KOH.

Media composition

Chemicals	Grams/litre

Peptone	**7**
KH_2PO_4	**5**
Dextrose	**5**

3.5.8 Nitrate reduction test

The capacity of the microorganisms to reduce nitrate to nitrite is recognized through the test. Microorganisms were inoculated into nitrate stock and incubated at 30±0.1°C for 96 hours. Sulphanillic acid and α-naphthyl amine mixture (1:1) was in added after the inoculation. Appearance of profound color demonstrated positive result. On the off chance that colour does not show up, the culture was diluted 2-5 fold and toasted once again.

Media Composition

Chemicals	**Grams/litre**
KNO_3	0.2
Peptone	5
pH	7.2

Reagent preparation:

Solution –A: sulphanic acid 8gm dissolved in 1litre of 5N acetic acid.

Solution –B: α- naphthylamine 5gm is dissolved in 1 litre 5N acetic acid.

Equal volume of solution A and B was mixed just prior to use.

3.5.9 Hydrogen sulphide (H_2S) production test

Hydrogen sulfide might be generated at any rate in little sums from sulfur holding amino acids by huge amount of bacteria. Techniques demonstrating hydrogen sulfide generation by suspending strips of paper impregnated with lead acetic above culture are of variable

affectability and are of constrained worth. Exact test most be poised at clear level of sensitivity. Hydrogen sulfide is exhibited by its capacity to form dark insoluble ferrous sulfide on the test strip or on the culture agar medium. The test strip ought to be ready by cutting white channel paper into strips more or less 5 by 50 mm, absorbing them a saturated solution of lead acetic, sanitizing in a plugged test tube and drying to a oven at 120°C. One of these strips ought to be replaced in the mouth of the culture before incubation in a position that one quarter to one a large portion of the strip projects below the cotton plug. The tubes were incubated at 20°C for no less than 7 days and the darkening of the strips were watched regularly day to day.

Media composition

Chemicals	Grams/litre
Peptone	10
Tryptone	10
Yeast	3
Beef extract	3
Maltose	10
Dextrose	1
Ferrous sulphate	0.2
NaCl	5
Phenol red	0.024
Agar	12

3.5.10 Starch hydrolysis test

The starch hydrolyzing limit of the microorganism is analysed the formation of basic substances like glucose, dextrin, maltose and so on the amylase compound is valuable to hydrolyze starch. The bacterial culture was inoculated on the nutrient agar medium with 1%

starch solution. The bacterial culture was inoculated on the nutrient agar media and it is incubated at 30±0.1°C for 24 hours. After culture development the iodine solution was flooded over the medium for five minutes. The excess solution was emptied and the hydrolysis of starch was examined as establishment of clear zone around the bacterial colonies. The hydrolysis of starch is shown by creation of reddish brown area.

Medium: Nutrient agar media + 1% soluble starch solution

Iodine solution: Iodine 1gm, potassium Iodide (KI) 2gm and water 300ml taken. Initially KI was dissolved in water and then I was added.

3.5.11 Triple Sugar Iron Test (TSI)

The medium holds three sugars to be specific: glucose, lactose and sucrose. Phenol red was utilized as a indicator and the analysis of hydrogen sulfide present was carried out by utilizing filter paper strips which were diped in the lead acetate. In the event if hydrogen sulfide was discharged by the culture of microscopic organisms could get dark. Agar slants were prepared ready for culturing of microorganisms and inoculation of culture was carried out by the method for stabbing media with the assistance of sterilized straight wire loop. Streaking of the culture was carried out by the loop. The culture was kept in incubation at 37^{o} C for 24 hrs after inoculation. 24 hrs of incubation was carried out. The generation of gas leads to the the breaking of the medium. The generation of gas was predicted by darkening of buffer at the slant butt intersection. The glucose fermentation was chosen by the butt slant to get yellow. The fermentation of lactose and sucrose was distinguished by the yellowing of the butts of slant media.

Media composition

Chemicals	Grams/litre
Peptone	10
Tryptone	10
Yeast	3
Beef extract	3
Maltose	10
Dextrose	1
Ferrous sulphate	0.2
NaCl	5
Phenol red	0.024
Agar	12

3.5.12 Mannitol Mortality Test:

Mannitol mortality agar was prepared and inoculated with the bacterial culture and incubated for 24 hours. Change of colour to yellow indicating positive reaction and no change indicated negative reaction. Presence of air bubbles was indicated nitrate positive and absence of air bubbles indicated nitrate negative. Heavy diffusion on stab agar was indicated mortality positive and simple growth was indicated mortality negative (7.8 for 30 minutes).

Media composition:

Chemicals	Grams/litre
Mannitol	15.0
Magnesium Sulphate	0.20
Di-potassium hydrogen Phosphate	0.5
Calcium Sulphate	0.1
Calcium Carbonate	5.0
Sodium Chloride	0.2

3.6 Identification of fungus by lactophenol cotton blue stain

Lactophenol blue solution is a mounting medium and staining executorutilized within the preparation of slides for microscopic examination of fungus. Fungal components are stained in deep blue colour. Place a drop of lactophenol blue dye on the slide. Utilizing an inoculation needle deliberately spread the fungal culture into a slim arrangement. Place a coverslip edge on the drop and gradually lower it. Avoid trapping air bubbles beneath the coverslip and wait for at least 5 min. If needed, seal the edges of the coverslip with nail shine or paramount to protect the mount as a kind of perspective slide. See under the microscopic lens with low power for screening in low intensity.

3.7 Study of Floral diversity

The assorted botanical qualities in and around the mining territory were done for floral study. The study was carried out on the basis of visual observation of plants depending upon leaves, reproductive parts of the plants i.e. flowers which includes stigma, ovary etc. and fruits.

Chapter 4

RESULTS AND DISCUSSION

4.1 Sampling area

Soil samples were collected from the eight geographical locations (Table 3) in and around the mining site in the state of Jharkhand. After collection of soil samples they were analyzed for further specifications.

Table 3: Geographic location of sampling sites recorded by GPS

Geographical locations	Longitude	Latitude
Ambajharan	84˚35’42”E	23˚49’569”N
Dhobijharan	84˚35’168”E	23˚49’206”N
Newari	84˚35’280”E	23˚49’206”N
Mangra	84˚35’519”E	23˚49’939”N
Dihi	84˚35’526”E	23˚49’689”N

Soil fruitfulness is a part of the soil plant relationship. Richness status of the soil is fundamentally and imperatively subordinate upon both the macro and micronutrient store of that soil. Proceeded evacuation of supplements by products, with almost no substitution will build the supplement stretch in plants and eventually brings down the benefit. The richness status of the soil primarily relies on upon the way of vegetation, atmosphere and geography, surface of soil and decay rate of natural matter. Ideal benefit of any editing frameworks relies on upon sufficient supply of plant supplements.

Figure 1: Collection of samples from different areas

4.2 Analysis of soil

Diverse soil samples were gathered from eight geographical locations as specified prior throughout August-September 2013 and were investigated for different physical and chemical properties. The physical property of the soil incorporates, shade, composition, texture, size, mass thickness, specific gravity and so on and the chemical properties incorporates, pH, moisture content, sulphur, chloride and phosphorus content.

4.2.1 Study of physical properties of soil samples

4.2.1.1 Color and texture:

Soil samples were examined and the color & texture were identified. The results were shown in Table 4.

Table 4: Color and texture of the soil samples

Sl.no.	Name of the village	Color	Texture
1	Dhobiajharan	Black	Clay
2	Mangra	Brown	Sandy
3	Newari	Black	Clayed loamy
4	Dihi	Brown	Sandy
5	Ambajharan	Blackish brown	Clay
6	Chope	Brown	Sandy clayed
7	Luti	Black	Clayed loamy
8	Murub	Black	Clay

4.2.2 Physical parameters of soil samples

Table 5: physical parameters of the soil samples

Sl.no	Name of village	PH	Moisture (%)	Bulk density (gm/cm^3)	Specific gravity
1	Mangra	7.52	4.66	0.97	1.047
2	Newari	7.47	13.73	0.942	1.01
3	Dhobijaran	7.36	14.44	1.002	1.07
4	Chope	7.34	10.7	1.202	1.29
5	Murub	7.31	14.31	0.901	0.97
6	Dihi	7.27	4.32	1.034	1.113
7	Luti	7.2	6.7	1.079	1.51
8	Ambajharan	6.95	26.18	0.859	0.92

4.2.2.1 pH

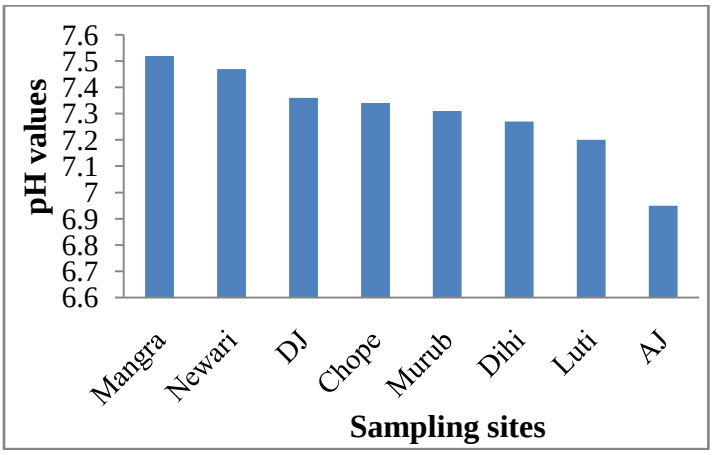


Figure 2: pH of the soil samples

The pH of Ambajharan site possesses 6.95 which is minimum compare with others and maximum pH 7.52 was found in Mangra. The pH range required for plant growth was 6.5 to 7.5. So all these samples having within required pH range which are suitable for growth of plants.

4.2.2.2 Moisture content

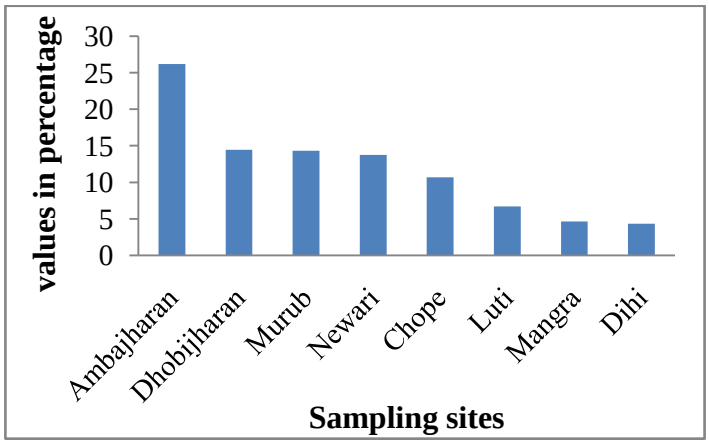


Figure 3: Moisture content of the soil samples

The identified moisture content was ranging from 4.32% to 26.18%. The maximum moisture

content 26.18% was found in Ambajharan and the minimum moisture content 4.32% was found in Dihi. Moisture content requirement for the growth of plants is varies from one plant another. These results indicated that all soil samples having less soil water (moisture).

4.2.2.3 Bulk density

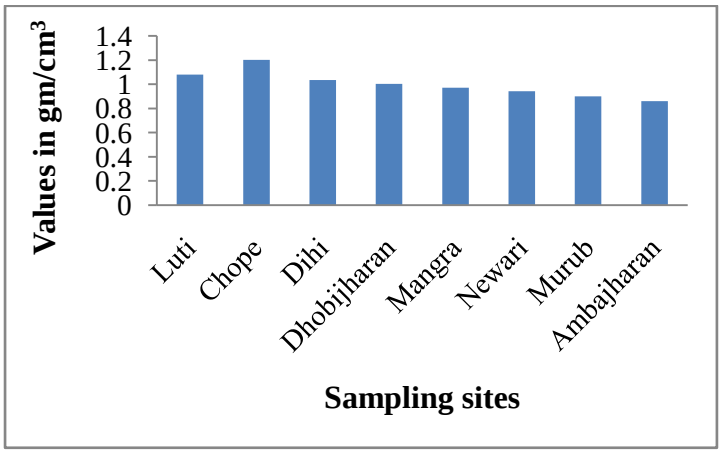


Figure 4: Bulk density of soil samples

The bulk density range is found to be 0.859 – 1.202 g/cm3. Ambajharan soil sample is having the minimum bulk density (0.859 g/cm3) and Chope having the maximum bulk density (1.202 g/cm3). The ideal bulk density is required for plant growth is 1.33 g/cm3. Sample containing high bulk density value cannot be used for vegetation and plantation growth. The soil samples are variable in their texture and the bulk density they contain is found optimal which is suitable for the growth of the plants. All soils having low bulk density which indicates the presence of higher organic content.

4.2.2.4 Specific gravity

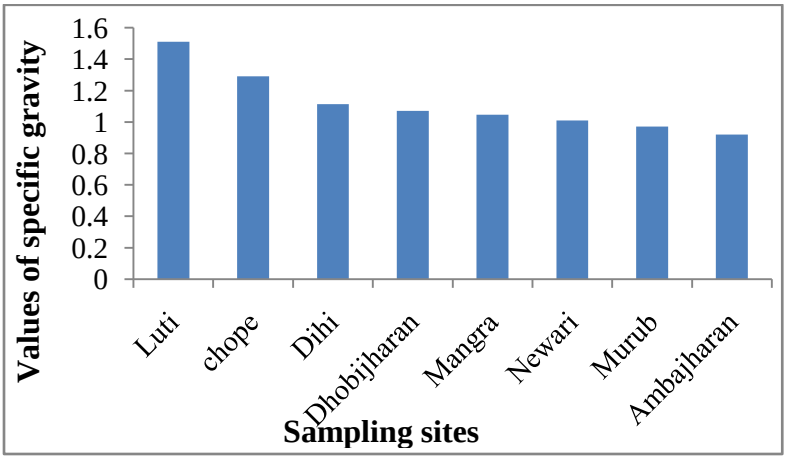


Figure 5: Specific gravity of the soil samples

The specific gravity range is found to be 1.21 to 1.56. The minimum specific gravity 1.21 is found in Newari and maximum 1.56 is found in Dhobiajharan. This specific gravity value is below 2 which mean all soil samples are containing high organic matter. Organic matter in soils is very essential for growth of plants.

Table 6: Normal ranges of specific gravity

Sl.no	Soil type	Specific gravity
1	Gravel	2.65-2.68
2	Sand	2.65-2.68
3	Silty sand	2.66-2.70
4	Silt	2.66-2.70
5	In organic clay	2.68-2.80
6	Organic Soils	Variable, may fall below 2

4.2.2 Study of chemical properties of soil sample

Table 7: Chemical properties of the soil sample

Sl.no.	Name of village	Chloride content (mg/g)	Phosphorus content (mg/g)	Sulphur content (mg/g)
1	Dihi	0.021	0.01	0.017
2	Murub	0.02	0.025	0.03
3	Dhobijharan	0.014	0.01	0.035
4	Ambajharan	0.012	0.015	0.067
5	Luti	0.011	0.005	0.021
6	Mangra	0.01	0.01	0.01
7	Newari	0.008	0.01	0.01
8	Chope	0.006	0.005	0.021

4.2.2.1 Chloride content

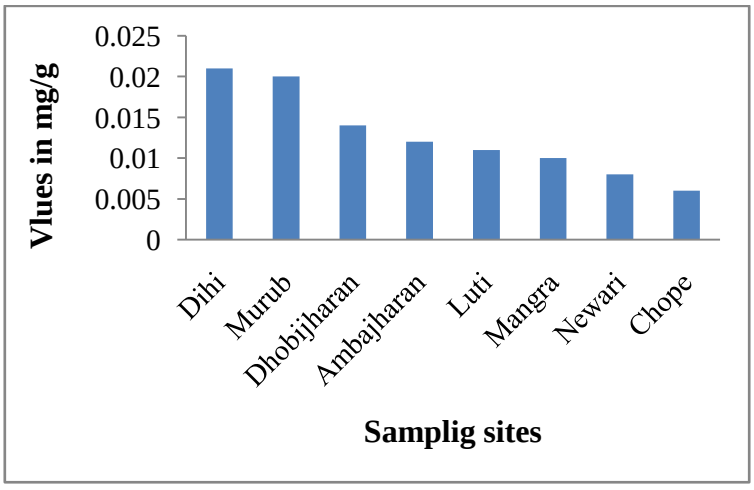


Figure 6: Chloride content of the soil samples

The chloride content range is found to be 0.006 to 0.021 mg/g. Chloride content maximum was present in Dihi and minimum was present in chope. Chloride is essential as a micronutrient for

optimal growth of the plant, at a range of only 0.3 – 1 mg/g dry matter to almost all plants (Marschner, 1986). In lithosphere the chloride content is almost 500 mg/ kg.

4.2.2.2 Phosphorus content

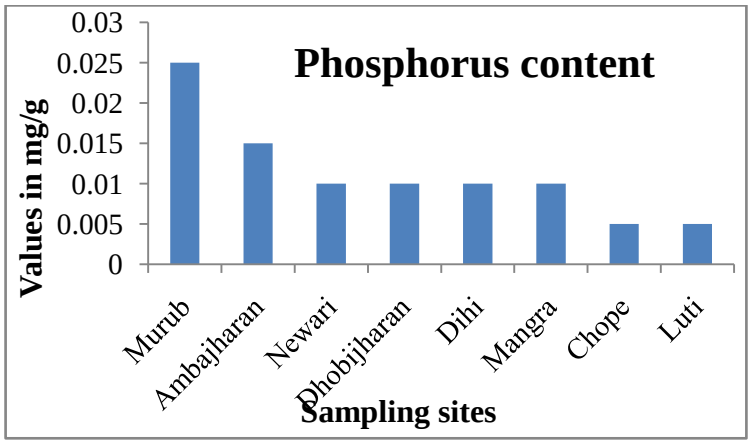


Figure 7: Phosphorus content of soil samples

Phosphorus content in soil samples was very low compare to normal ranges. Maximum 0.025 mg/g was found in Murub and minimum 0.005mg/g was present in Luti. Both the soils contain less than normal range of phosphorus. Acceptable Phosphorus availability for plants was there which can encourage early plant growth and quicken maturity. Although Phosphorus is very important for plant growth, mismanagement of soil P can pose a threat to water quality.

4.2.2.3 Sulphur content

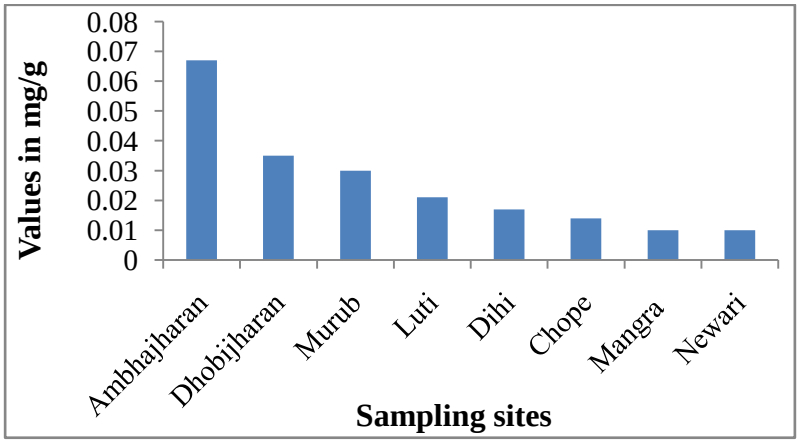


Figure 8: Sulphur content of soil samples

The sulphur content measured was found to be maximum in the Ambajharan is 0.067 mg/g and to be minimum was found in mangra and newari that is 0.01 mg/g. the sulphur content was found to be low in the soil.

4.2.3 Microbial diversity

The soil specimens were screened for the microbial load as far as bacterial burden exhibit in them. The aggregate number of bacterial load in colony forming unit was additionally recorded by the standard plate count strategy. Determination of fungi and actinomycetes load in the soil and water samples have also been done. These microscopic organisms vicinity demonstrates numerous types of supplements are available in the soil and water which direct the growth. Soil richness additionally relies on upon soil microbial diversity. Some nitrogen fixing microorganisms give nitrogen to leguminous plants. This symbiotic cooperation is required one another as plant offers shelter to microorganisms and in turn microorganisms give supplements to plants. Determination of bacterial burden was carried out by utilizing Nutrient Agar medium

and determination of fungal load was carried out by using potato dextrose agar and the result was introduced in Table 8 & Table 10.

Table 8: Determination of Bacterial load in the soil samples

Sample	Dilution factor	No. of colonies	Organisms per gram of soil (CFU/gm)
Ambajharan	10^{-6}	68	68×10^{-6}
Dhobijharan	10^{-6}	63	63×10^{-6}
Mangra	10^{-6}	79	79×10^{-6}
Dihi	10^{-6}	77	77×10^{-6}
Newari	10^{-6}	71	71×10^{-6}
Chope	10^{-6}	55	55×10^{-6}
Murub	10^{-6}	85	85×10^{-6}
Luti (river)	10^{-6}	31	31×10^{-6}

Table 9: Morphological characterization of Bacterial samples isolated from the soil samples

Name of geographical locations	Bacteria code	Forms	Size (cm)	Surface	Texture	Color	Elevation	Margin
Ambajharan	A1	Circular	Punctiform	Smooth & shiny	Moist	Cloudy	Raised	Entire
Dhobijharan	DH1	Circular	0.1	Smooth & shiny	Moist	Brownish	Raised	Entire
Mangra	MA1	Circular	0.2	Rough	Dry	Brownish	Raised	Entire
Dihi	DI1	Circular	0.2	Smooth & shiny	Moist	Cloudy	Convex	Entire
Newari	N1	Circular	0.2	Rough	Dry	Cloudy	Raised	Entire
	N2	Irregular	0.5	Smooth & shiny	Moist	Opaque	Pulvinate	Curled
Chope	C1	Circular	0.1	Rough	Dry	Cloudy	Raised	Entire

	C2	Irregular	0.5	Smooth & shiny	Moist	Brownish	Convex	Undulate
Murub	M1	Circular	Punctiform	Smooth & shiny	Moist	Cloudy	Raised	Entire
	M2	Irregular	0.3	Rough	Dry	Brownish	umbonate	Undulate
Luti (river)	L1	Circular	0.2	Smooth & shiny	Moist	Brownish	Raised	Entire
	L2	Irregular	0.3	Rough	Dry	Brownish	umbonate	Undulate

Table 10: Determination of bacterial load in the water samples

Sample	**Dilution factor**	**No. of colonies**	**Organisms per gram of water (CFU/gm)**
Ambajharan	10^{-6}	202	202×10^{-6}
Murub (well)	10^{-6}	39	39×10^{-6}
Kundri	10^{-6}	161	161×10^{-6}
Murub (pond)	10^{-6}	128	128×10^{-6}
Dhobijharan	10^{-6}	124	124×10^{-6}
Mangra (handpump)	10^{-6}	56	56×10^{-6}
Dihi	10^{-6}	72	72×10^{-6}
Newari	10^{-6}	238	238×10^{-6}
Chope	10^{-6}	184	184×10^{-6}

Table 11: Morphological characterization of Bacterial samples isolated from the water sample

Name of geographical locations	Bacteria code	forms	Size (cm)	Surface	Texture	Color	Elevation	Margin
Ambajharan	A1	Circular	0.3	Smooth & shiny	Moist	Cloudy	Flat	Entire
	A2	Irregular	0.7	Rough	Moist	Brownish	Flat	Undulate
Murub (well)	MW1	Circular	0.4	Rough	Moist	Cloudy	Flat	Entire
	MW2	Irregular	0.5	Smooth & shiny	Moist	Opaque	Flat	Undulate
Kundri	K1	Circular	0.2	Rough	Moist	Cloudy	Raised	Entire
	K2	Irregular	0.4	Smooth & shiny	Moist	Cloudy	Flat	Undulate
Murub (pond)	MP1	Circular	0.3	Rough	Moist	Cloudy	Convex	Entire
	MP2	Irregular	0.5	Smooth & Shiny	Moist	Opaque	Flat	Undulate
	DH1	Circular	0.2	Rough	Moist	Cloudy	Convex	Entire

Dhobijharan	DH2	Irregular	0.4	Smooth & shiny	Moist	Brownish	Flat	Undulate
Mangra (handpump)	MH1	Circular	0.3	Rough	Moist	Cloudy	Convex	Entire
	MH2	Irregular	0.3	Smooth & shiny	Moist	Brownish	Flat	Undulate
Dihi	DI1	Circular	0.2	Smooth & shiny	Moist	Cloudy	Umbonate	Entire
	DI2	Irregular	0.2	Rough	Moist	Translucent	Flat	Undulate
Newari	N1	Irregular	0.2	Rough	Moist	Translucent	Flat	Curled
	N2	Circular	0.2	Smooth & shiny	Moist	Brownish	Raised	Entire
Chope	C1	Circular	0.2	Smooth & shiny	Moist	Cloudy	Convex	Entire
	C2	Irregular	0.7	Rough	Moist	Brownish	Flat	Undulate

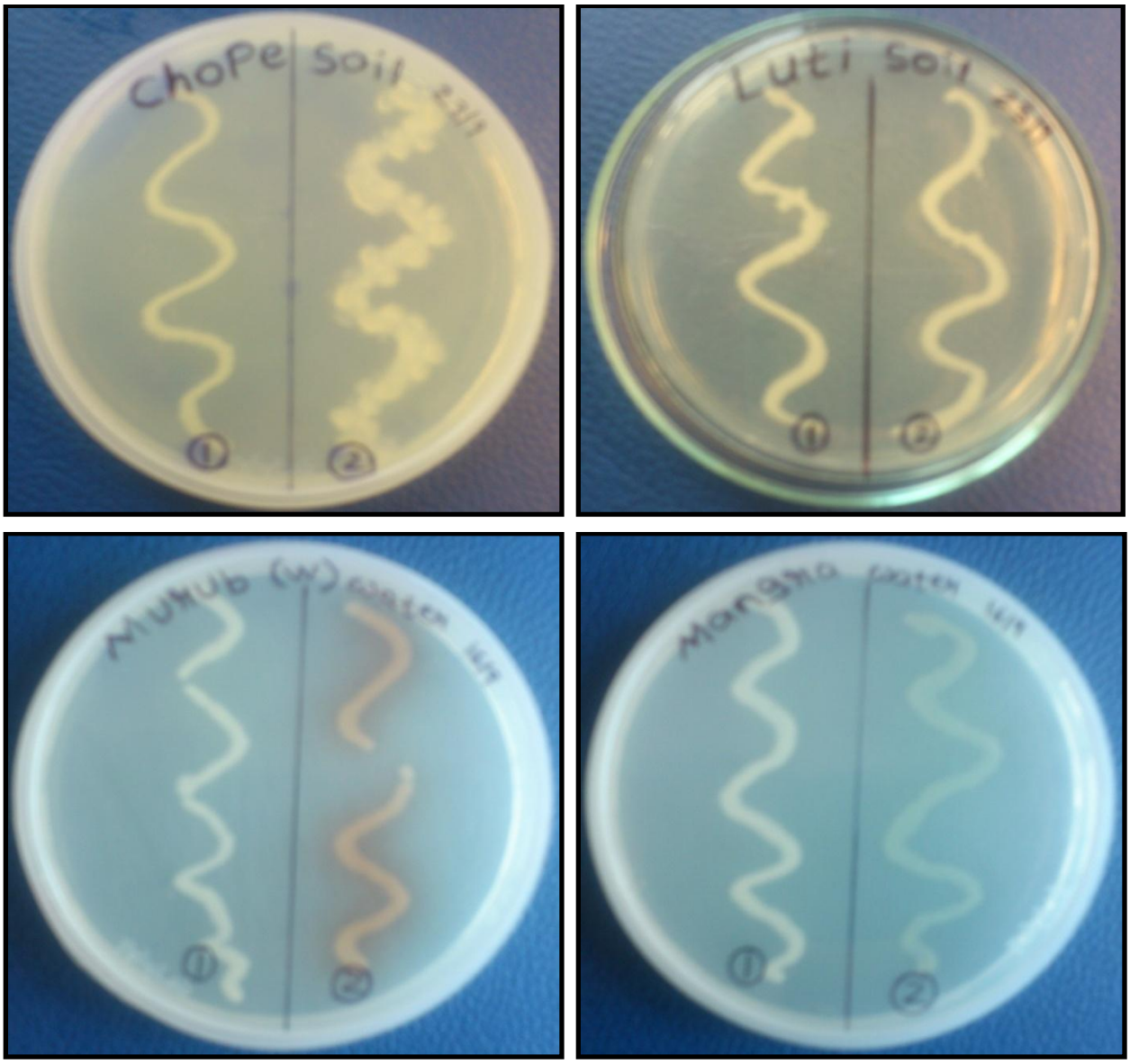


Figure 9: Morphological characteristics of isolated bacteria from soil and water samples

It is observed that luti showed pale coloured colony and chope showed two different type of bacterial colonies from soil sample. Murub showed two types of colony one is cloudy white and other is brown in colour and mangra showed two types of colonies one is cloudy white and other is translucent colony.

Table 12: Morphological characterization of fungal samples isolated from the soil samples

Name of geographical locations	Form	Size (cm)	Surface	Texture	Color	Elevation	Margin
Chope	Irregular	2.2	Rough	Dry	Black	Raised	Undulate
Murub	Irregular	0.4	Rough	Dry	White	Raised	Undulate
Luti	Circular	0.4	Rough	Dry	White	Raised	Entire
Dhobijharan	Irregular	0.4	Rough	Dry	Creamy	Raised	Undulate
Newari	Circular	0.4	Rough	Dry	White	Raised	Entire
Ambajharan	Irregular	0.5	Rough	Dry	White	Raised	Wavy

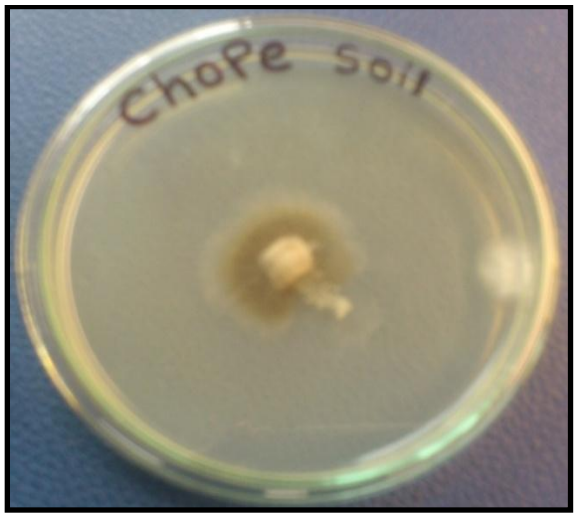


Figure 10: Morphological characteristics of isolated Fungus from soil and water sample

It is observed that the fungus of dhobijharan is brown in color, fungus in chope is blackish in colour from soil samples. The fungus from chope and ambajharan from water samples are white in colour.

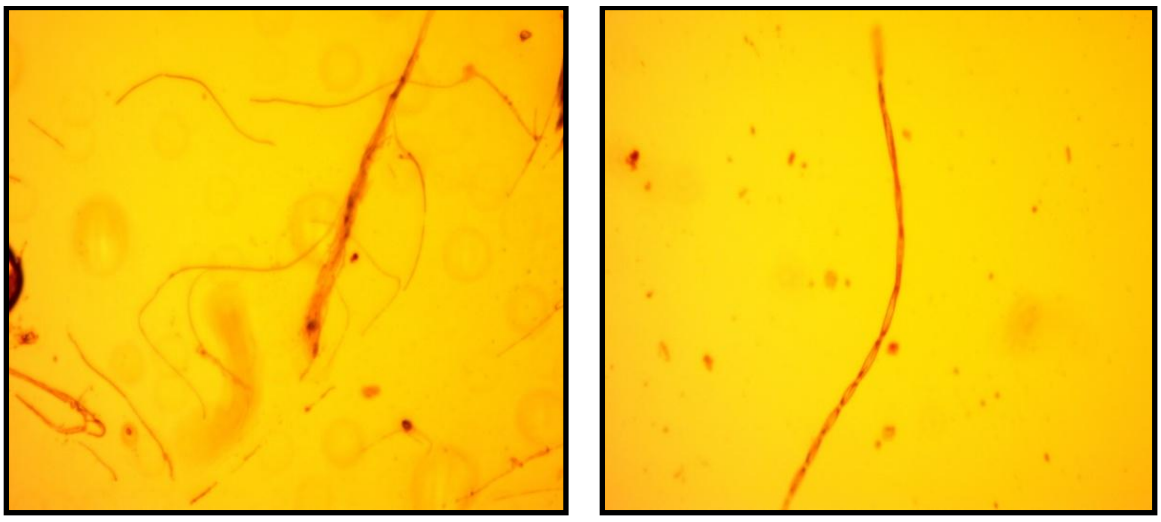


Figure 11: Lactophenol cotton blue staining of isolated fungus from soil samples

The fungus specimens isolated from the soil and water samples of different geographical locations are distinguished as filamentous (*Aspergillus spp.)* in nature. Characteristics of

Filamentous fungi: Hyphae are septate and hyaline. The septate hyphae are separated into two sections divided by cross walls, each section contains one or more nuclei. The conidiophores begin from the basal foot cell found on the supporting hyphae and end in a vesicle at the zenith. The morphology and shade of the conidiophore change starting with one animal varieties then onto the next. Coating the surface of the vesicle totally ("transmit" head) or halfway just at the upper surface ("columnar" head) are the jar molded phialides which are both uniseriate and joined to the vesicle straightforwardly or are biseriate and connected to the vesicle through a supporting cell, metula. Over the phialides are the round conidia (2-5 μm in measurement) shaping spiral chains.

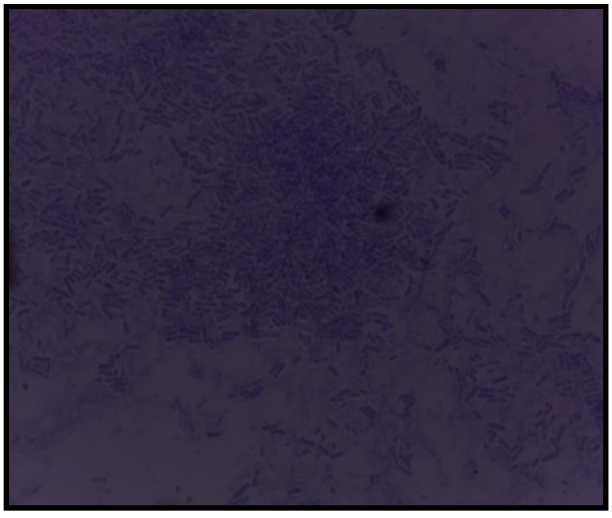
4.2.4 Biochemical characterization of bacterial samples

Biochemical tests were performed and the results were presented in Table 13 and Figures 11-23 below.

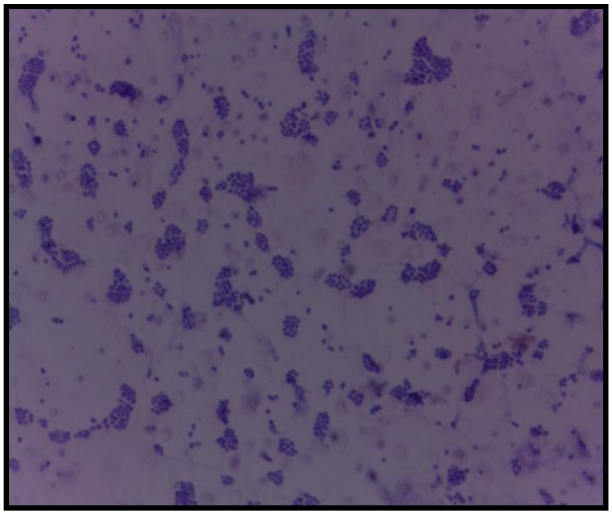
Table 13: Biochemical tests results for isolated bacteria form top soil samples of study site

Biochemical tests	**Murub (Water)**	**Ambajharan (Water)**	**Chope (Water)**	**Mangra (Water)**	**Ambajharan (Soil)**	**Luti (Soil)**	**Murub (Soil)**
Grams staining	-ve	+ve	-ve	-ve	+ve	-ve	+ve
Catalase Test	-ve	+ve	+ve	-ve	+ve	-ve	-ve
Methyl red Test	+ve	-ve	-ve	+ve	+ve	+ve	+ve
Urease Test	+ve	-ve	-ve	+ve	+ve	+ve	+ve

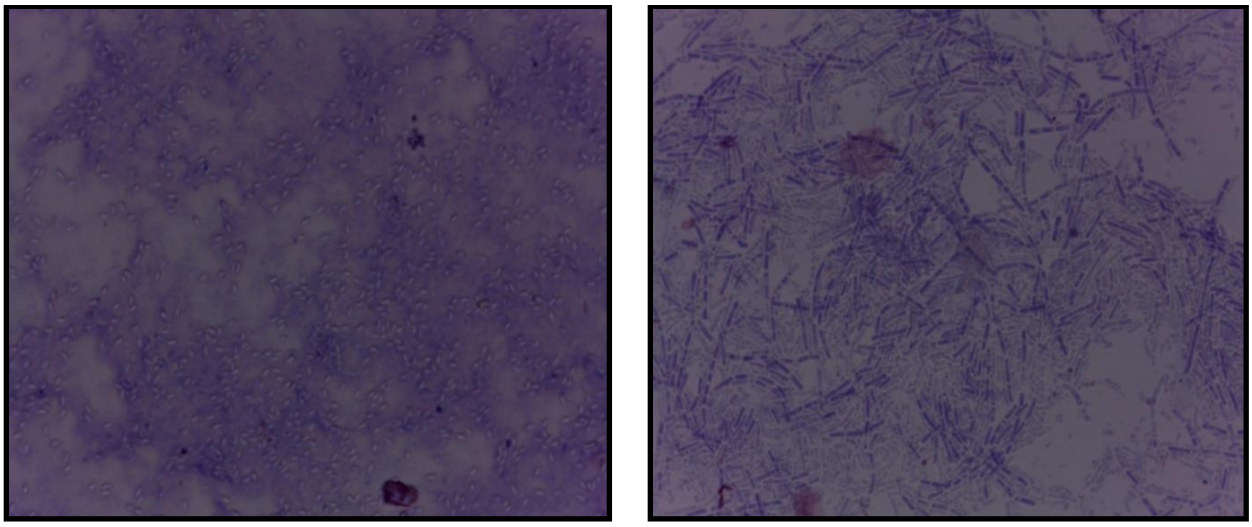
Citrate Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Indole Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Nitrate Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Oxidase Test	+ve	-ve	-ve	+ve	+ve	+ve	+ve
Starch Hydrolysis test	-ve	-ve	-ve	+ve	-ve	+ve	+ve
Voges proskea-ur Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Manitol mortality Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Triple sugar Iron test	+ve	-ve	-ve	+ve	+ve	+ve	+ve
H2S gas Production test	-ve	+ve	+ve	-ve	-ve	-ve	-ve



Lactobacillus spp.



Staphylococcus spp.



Aeromonas spp. ***Corynebacterium spp.***

Figure 12: Gram staining of isolated bacteria from soil samples

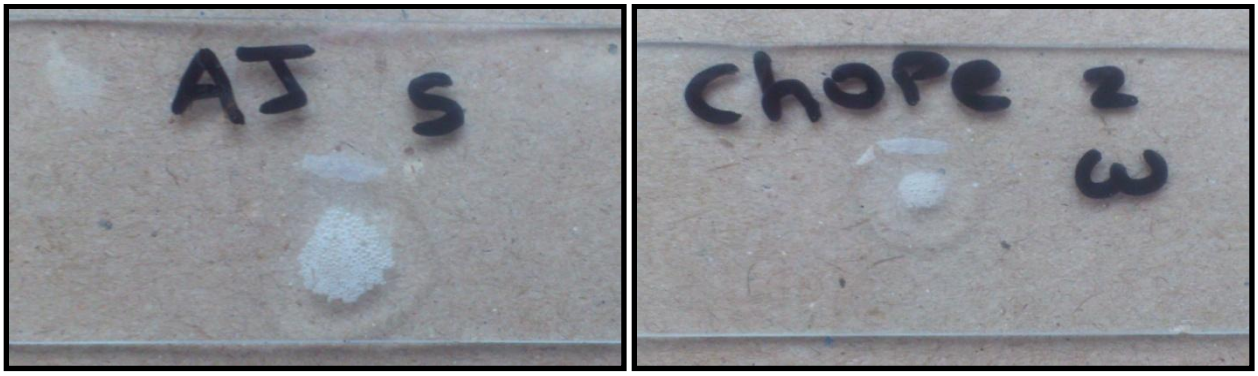


Figure 13: Catalase test of isolated bacteria from soil & water samples

Ambajharan (soil sample) and chope (water sample) showed presence of bubbles which indicated presence of catalase enzyme in microorganisms showing positive result.

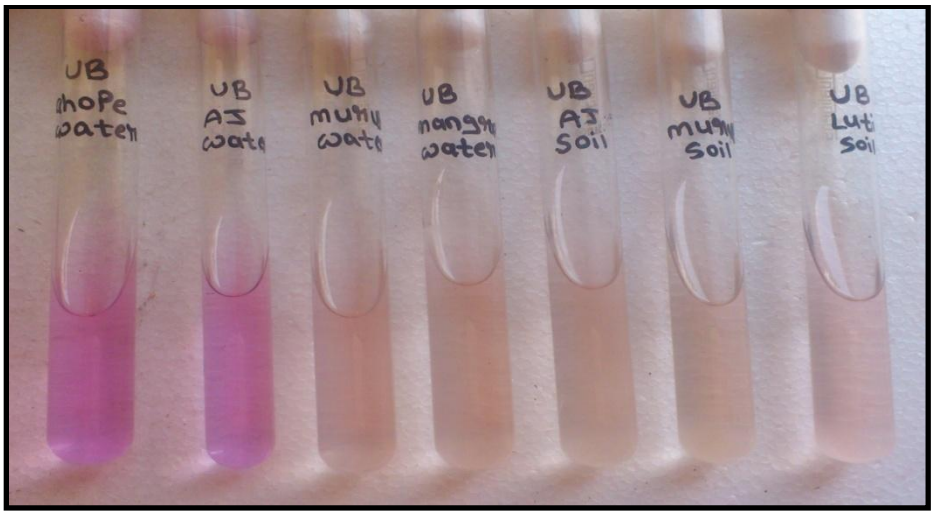


Figure 14: Urease test of isolated bacteria from soil & water samples

The Ambajharan and chope (water samples) showed negative result for the test which are purplish pink in colour. The colourless test tubes showing positive result.

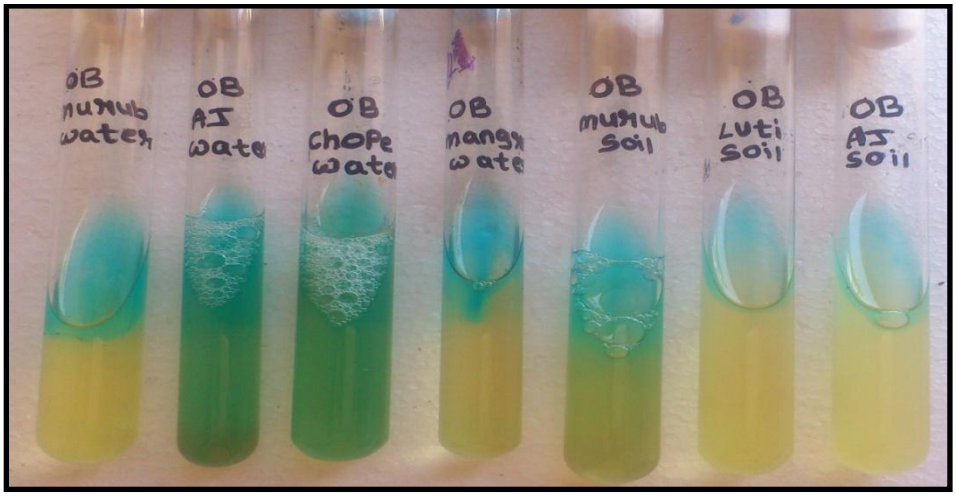


Figure 15: Oxidase test of isolated bacteria from soil & water samples

The Ambajharan and Chope (water samples) showed negative result for the test which are in green colour. the colourless test tubes showing positive result.

Figure 16: citrate test of isolated bacteria from soil & water samples

All the samples showed negative result in the test. The formation of deep blue color after incubation shows a positive result. No growth and yellowish-green color of the slant showed negative result.

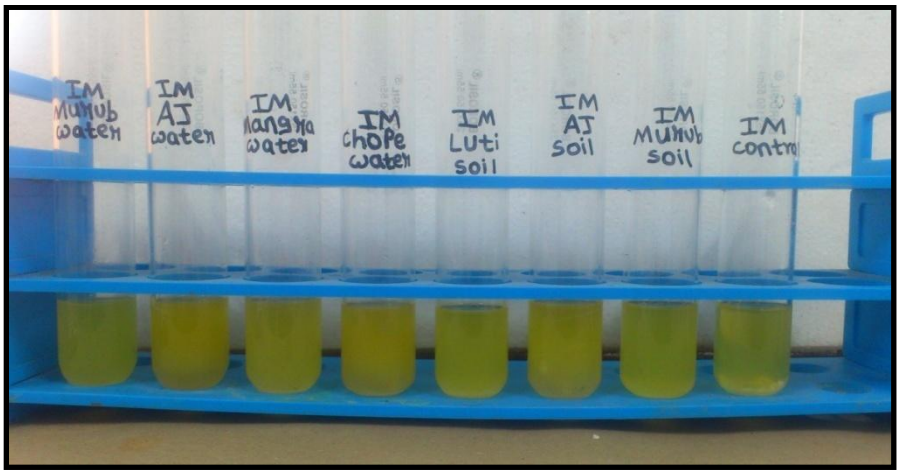


Figure 17: Indole test of isolated bacteria from soil & water samples

All the samples showed negative result in the test. The formation of red coloration at the top layer showed positive and yellow coloration demonstrates negative.

Figure 18: Mannitol mortality test of isolated bacteria from soil & water samples

All the samples showed negative results in the test. Changing of colour to yellow indicated positive reaction and no change indicated negative reaction.

Figure 19: Methyl red test of isolated bacteria from soil & water samples

Ambajharan and chope (water samples) showing negative response while the test tube which are in red colour showed positive response.

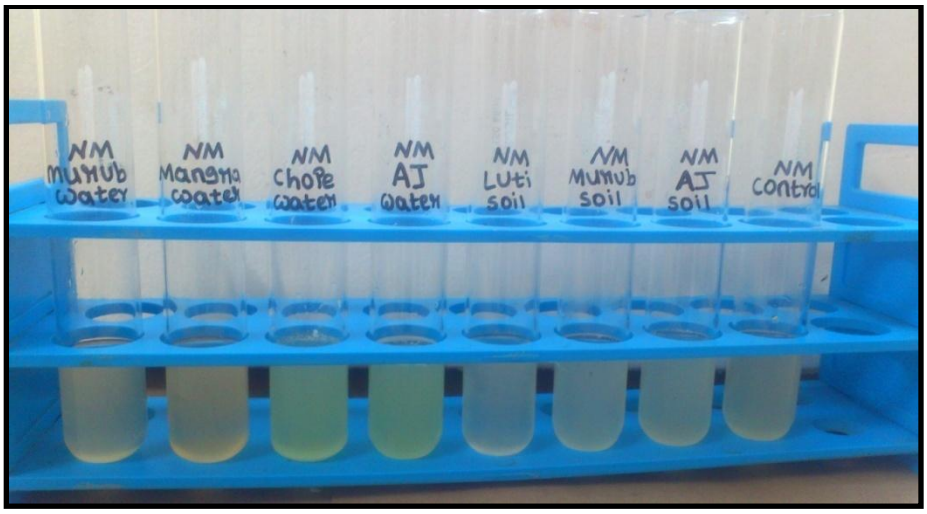


Figure 20: Nitrate test of isolated bacteria from soil & water samples

All the samples showed negative results. Appearance of deep color demonstrated positive result. On the off chance that colour does not show up, the culture was diluted 2-5 fold and toasted once again.

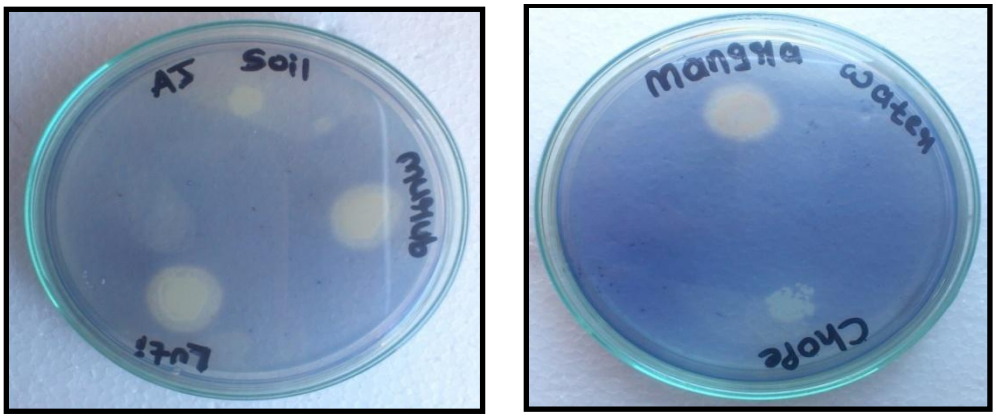


Figure 21: Starch hydrolysis test of isolated bacteria from soil & water samples

Ambajharan, luti and murub (soil samples) are showing positive response for hydrolysis of starch, this means starch hydrolyzing microorganisms are present in these samples. While mangra, chope showing positive response in water samples. The hydrolysis of starch is shown by creation of reddish brown area.

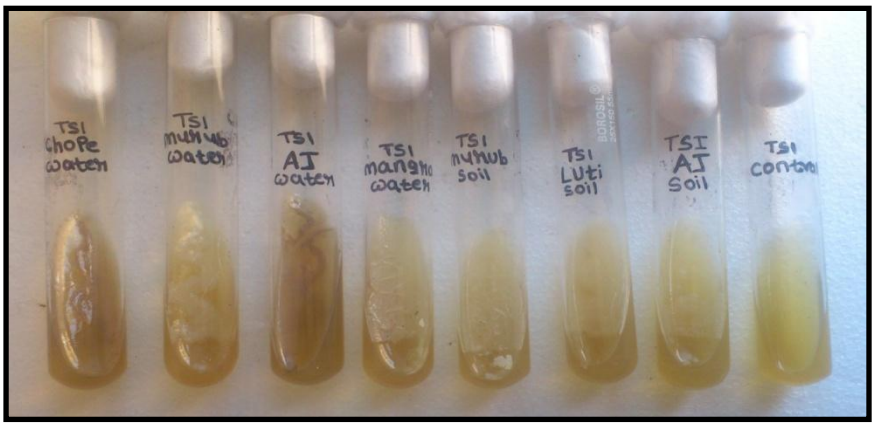


Figure 22: Triple sugar iron and H_2S gas production test of isolated bacteria from soil & water samples

The samples chope and ambajharan showed positive response for H_2S gas production while other samples are positive for glucose fermentation. The generation of gas leads to the the breaking of the medium. The generation of gas was predicted by darkening of buffer at the slant butt intersection. The glucose fermentation was chosen by the butt slant to get yellow.

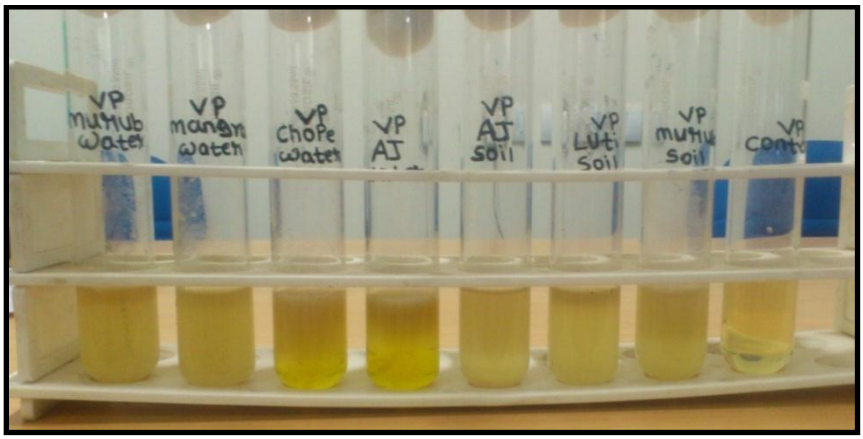


Figure 23: Voges-progskaeur test of isolated bacteria from soil & water samples

The voges-progskaeur test of the sample showed that all the samples are negative for the test. Presence of dark red color of the medium demonstrated positive result.

4.2.5 Identification of unknown bacterial species

Identification of bacteria was done according to Bergey's Manual of Determinative Bacteriology [41] by using various biochemical test results. The bacteria identified are presented in the table 20.

Table 14: Identification of bacterial species isolated from top soil and water samples

Biochemical tests	Murub (Water)	Ambajharan (Water)	Chope (Water)	Mangra (Water)	Ambajharan (Soil)	Luti (Soil)	Murub (Soil)
Grams staining	-ve	+ve	-ve	-ve	+ve	-ve	+ve
Catalase Test	-ve	+ve	+ve	-ve	+ve	-ve	-ve
Methyl red Test	+ve	-ve	-ve	+ve	+ve	+ve	+ve
Urease Test	+ve	-ve	-ve	+ve	+ve	+ve	+ve
Citrate Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Indole Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Nitrate Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Oxidase Test	+ve	-ve	-ve	+ve	+ve	+ve	+ve
Starch Hydrolysis test	-ve	-ve	-ve	+ve	-ve	+ve	+ve
Voges proskea-ur Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Manitol mortality Test	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Triple sugar Iron test	+ve	-ve	-ve	+ve	+ve	+ve	+ve
H2S gas Production test	-ve	+ve	+ve	-ve	-ve	-ve	-ve
Bacterial species identified	*Aeromonas spp.*	*Corynebacterium spp.*	*Neisseria spp.*	*Aeromonas spp.*	*Staphylococcus spp.*	*Aeromonas spp.*	*Lactobaacillus spp.*

4.2.6 Study of Flora

Table 15: Floral species found at the study site

Sl.no	Common name	Scientific name
1	Wild Date Palm tree	*(Phoenix dactylifera)*
2	Teak	*(Tectona grandis)*
3	Thumbai	*(Leucas aspera)*
4	Flea Tree	*(Albizea lebbeck)*
5	Jamun	*(sygizium cuminii)*
6	Mahua	*(Madhuca longifolia)*
7	Kachnar	*(Bauhinia racemosa)*
8	Tendu	*(Diospyros melanoxylon)*
9	Sarpagandha	*(Rauvoulfia serpetina)*
10	Bamboo	*(Bambuseae)*
11	Capsicum	*Capsicum pubescens*
12	Palash	*Butea monosperma*
13	Wild sage	*Lantana camara*

14	Radhachura	*Peltophorm pterocarpm*
15	Lion's ear	*Leonotis nepetifolia*

Table 16: Plant types and optimum soil characteristics

Area	**Soil characteristics (mg/g)**	**Plant present**	**Suggested plants**
Mangra (79.62 acre)	Sandy (Black) PH- 7.52 Sulphur- 0.01 Phosphorus-0.01 Chloride-0.01	*Diospyros Melanoxylon, Madhuca longifolia*	*Azadirachta indica, Swietenia mahagoni, Casuarina junghuhniana*
Newari (86.15 acre)	Clayed loam (black) pH- 7.47 Sulphur-0.01 Phosphorus-0.01 Chloride-0.008	*Bambuseae, Capsicum pubescens, Bauhinia racemosa*	*Shorea robusta, Moringa oleifera*
Ambajharan (280.42 acre)	Clay (blackish brown) pH- 6.95 Sulphur- 0.067 Phosphorus-0.015 Chloride-0.012	*Tectona grandis, Butea monosperma*	*camellia sinensis, Melia dubia*
Chope (57.8 acre)	Sandy clayed (brown) pH- 7.34 Sulphur- 0.014 Phosphorus-0.005 Chloride-0.006	*Syzygium cumini , Albizia lebbeck*	*Warburgia ugandensis, Vitex pubescens, Tamarindus indica*
Dhobijharan (312.29 acre)	Clay (black) pH- 7.36 Sulphur- 0.035 Phosphorus- 0.01 Chloride-0.014	*Phoenix dactylifera*	*Senna siamea, Gmelina arborea, Bambusa nutans*
Dihi (126.82 acre)	Sandy (brown) pH- 7.27 Sulphur-0.017 Phosphorus-0.01 Chloride-0.021	*Rauvolfia serpentine*	*Eucalyptus tereticornis, Pinus wallichiana, Ailanthus excels, Leucas aspera,*
Murub (60.5 acre)	Sandy (black) pH- 7.31 Sulphur- 0.03 Phosphorus-0.025	*Lantana camara, Peltophorm pterocarpm, Leucas aspera*	*Dalbergia sissoo, Thespesia populnea, Delonix regia, Ficus religiosa*

	Chloride- 0.02		
Luti (river)	Clayed loam (black) pH- 7.2 Sulphur- 0.021 Phosphorus-0.005 Chloride-0.011	*Leonotis nepetifolia*	*Zea mays, Paulownia tomentosa, Artocarpus heterophyllus, Azadirachta indica*

4.2.6.1 Mitigation measures for flora

- The trees ought to be checked by taxonomist and examined before cleaning [42] .
- Collect appropriate reach of by regional standards indigenous plant seed for utilization in revegetation.
- Minimize extraordinary plant habitat loss by keeping local seeds for future estate [43].
- Compaction of soil and trampling of tree roots by machine may prompt harm and death of reatained trees and ought to be kept away from.
- Key framework, for example, stockyards, rail circle, foundation passageway and exchange station, has been located in or adjacent to aggravated zones [44].
- Trees that have been recognized as suitable for giving local seed source are checked and recorded for further utilize [45].
- Unique plants are found in with potential for translocation [46].
- Topsoil stripping and the ideal utilization of local top soil throughout restoration is key part of rehabilitation.

Chapter 5

CONCLUSION

The analysis of top soil and water were collected from the coal mining area exhibit that the pH of soil is slightly basic in nature (range 6.95-7.52),, which found to be optimal for growth of the plants. The bulk density and specific gravity of all the soil samples were found to be very low so it indicates that the soil has high organic matter which is favourable for the growth of the plants in this soil mainly in Ambajharan, Murub, Newari, Mangra and low in Chope. The chloride content is high in dihi where as low in chope. However the moisture content is quite low in all the areas under study. Phosphorus content of all the soil sample was found to be in the normal range. The vicinity of a various groups of microorganisms shows that the soil is rich in all kind of macro and micro supplements suitable for their development.

From the result it is inferred that the soil is rich in macronutrients which is crucial for the development of plants and in addition to the microorganisms. Five bacterial isolated were identified from the soil and water samples: .*Aeromonas spp.* which are a gram-negative, non spore forming and are facultative anaerobic rods that morphologically take after parts of the family Enterobacteriaceae. *Corynebacterium spp.* are a family of Gram-positive, non spore forming, non motile rod shaped microbes. *Neisseria spp.* are Gram-negative microscopic organisms included among the proteobacteria. *Staphylococcus spp.* is a family. It is Gram-positive microorganisms. *Lactobaacillus spp*. are a class of Gram-positive facultative anaerobic or microaerophilic bar formed bacteria and ane fungal (aspergillus spp.) Consequently the suitability of the soil ought to be kept up like land rehabilitation or mine recovery after fulfillment of mining in the proposed range. Some mitigation measures must be embraced by the mining organization to monitor and safeguard the characteristic property of the soil, plants and animals living there. The Mitigation measures were suggested to keep the flora safe and fulfil the future requirement for the land reclamation. 15 plant species have been studied from the studied

site and mitigation measures were studied to conserve and preserve the natural property of the soil and plants.The plants have been suggested for the land reclamation after mining which can be planted in that area according to their soil type, which depends on the soil properties.

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